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Older males attract more females but get fewer matings in a wild field cricket

Rolando Rodríguez-Muñoz [a], Paul Hopwood [a], David Fisher [a, d], Ian Skicko [a], Rachel Tucker [b], Katherine Woodcock [b], Jon Slate [b], Craig Walling [c], Tom Tregenza [a, *]

[a] Centre for Ecology & Conservation, School of Biosciences, University of Exeter, Penryn Campus, Penryn, UK
[b] Department of Animal & Plant Sciences, The University of Sheffield, Sheffield, UK
[c] Institute of Evolutionary Biology, University of Edinburgh, Ashworth Laboratories, Edinburgh, UK
[d] Department of Psychology, Neuroscience & Behaviour, McMaster University, Hamilton, ON, Canada

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The age of potential mates has been proposed to be an important target for mate choice by females. Alternative hypotheses predict preferences in either direction. Females might be expected to prefer older males because such males have demonstrated their capacity to survive. Alternatively, they might prefer younger males that have not accumulated deleterious mutations. Preferences in both directions have been observed in laboratory experiments, suggesting that this is an issue that needs to be understood within its ecological context. We measured individual behaviour and reproductive success in a natural population of the field cricket *Gryllus campestris* over 10 years. We found that in this annual insect, a male's age relative to his peers was poorly correlated with his life span. This suggests that there is limited potential for selection to favour female choice for older males because a strategy of choosing older males would not significantly increase a female's likelihood of mating with a long-lived male. Older males were more successful at pairing up with females at a burrow, but once paired they were less likely to mate with them. By genotyping the next generation of adults we confirmed that observations of both pairing up with a female and matings were associated with successful offspring production. However, there was no relationship between how old a male was at mating and how many adult offspring he had. This lack of evidence for any fitness benefits to females from mate choice in relation to male age was consistent with the observation that the age of males had opposite effects on their success in pairing up with females compared to their success in mating with them.

Females are expected to exercise choice among potential mates if that choice leads to higher quality offspring. One male trait on which females may base these decisions is the age of a potential mate. However, there are contrasting (but not exclusive) predictions regarding potential advantages or disadvantages for females choosing older or younger males as mates. Germ-line mutations accumulated during a male's life have the potential to lead to declines in fertility and offspring viability (Johnson & Gemmell, 2012). Such declines should select for avoidance of mating with older males in order to maximize fertilization and development of females' eggs (Aitken & De Iuliis, 2007; Beck & Promislow, 2007; Preston, Saint Jalme, Hingrat, Lacroix, & Sorci, 2015). In contrast, females may benefit from choosing older mates if survival to old age is indicative of genetic quality, which will be inherited by their offspring (Brooks & Kemp, 2001; Kokko & Lindström, 1996). These two opposing selection pressures will act simultaneously, with the expectation being that female preferences will reflect the balance between costs and benefits of mating with older or younger males.

Organisms tend to exhibit physiological deterioration with age, a process known as senescence (Rodríguez-Muñoz, Boonekamp, Liu, Skicko, Haugland Pedersen, Fisher et al., 2019; Rose, 1991). This deterioration is generally thought to be the result of trade-offs between somatic maintenance and investment in reproduction (Kirkwood & Holliday, 1979; Partridge & Barton, 1993; Rodríguez-Muñoz, Boonekamp, Liu, Skicko, Fisher, Hopwood et al., 2019; Williams, 1957). In this context there is a clear prediction that individuals that are better adapted to their environment or that carry

* Correspondence: Centre for Ecology and Conservation, University of Exeter, Penryn Campus, Penryn, TR10 9FE, U.K.
E-mail address: T.Tregenza@exeter.ac.uk (T. Tregenza).

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other forms of 'good genes' should be able to sustain increased costs of both somatic and reproductive investment. As a result, these individuals are expected to have a greater capacity to survive to an old age. Consequently, females who preferentially mate with older males should increase the probability that their eggs will be fertilized with sperm carrying genes that confer high fitness on their offspring (Andersson, 1994; Trivers, 1972). However, the predictions made by models examining the value of old age as an indicator of high quality depend on the assumptions upon which they are built. Hansen and Price (1995) dismissed the potential role of age as an indicator of high genetic quality. They invoked potential declines in fertility and increases in mutation load with age (as discussed above) and argued that survival to old age is not an indicator of genetic quality. In contrast, a simulation model by Kokko and Lindström (1996) supported the possibility of age acting as a good-genes indicator. This latter conclusion seems more likely to be correct, as discussed by Kokko (1998), who demonstrated that Hansen and Price's (1995) model did not allow for differences in quality between males. Nevertheless, Kokko and Lindström's (1996) finding relied on large differences in genetic quality between males expressed in their life span. In such a case, females can benefit from a mating rule that makes them increasingly likely to mate with a male according to his age. However, in assessing the likely importance of such a mechanism, note that although older males are known to be long-lived, young males may also go on to be long-lived. Hence the strength of the correlation between the age of a male relative to his peers and how long he lives may not be as strong as it intuitively appears to be. This is an issue that becomes particularly important in populations where the assumption that there is a stable age distribution is not met, for instance in annual organisms with a single breeding season.

Across taxa, populations vary from those in which males continually enter the adult population over the course of 2 years or more, as in most vertebrates, to those with discrete generations in which males become adult during a brief annual time window (Śniegula, Gołąb, & Johansson, 2016). This variation in seasonal life history determines how accurately age reflects life span. At one extreme, if all males are born simultaneously, they will all have the same age, so at any point in time when a female is choosing a mate, the age of the males available to her will have no relationship to their longevity. Annual species, such as most temperate insects where adults emerge during a short time window, are not quite at this extreme, but nevertheless, the relative age of the males available as mates will provide a more limited amount of information about their longevity than in continuously reproducing populations. Females could delay mating in order to sample only old males, but they risk dying and deteriorating in the meantime and they would have to delay producing offspring. Furthermore, delaying mating would select for males that emerge later, which might reduce the power of delaying mating as a strategy to select for longer-lived adult males.

Species where there is high variance in the date of birth of males and in their longevity include many birds (Akçay & Roughgarden, 2007), for example great bustards, *Otis tarda* (Alonso, Magaña, Palacín, & Martín, 2010), numerous large mammals, for example Soay sheep, *Ovis aries* (Clutton-Brock & Pemberton, 2004), and many reptiles, including *Lacerta monticola* (López, Aragón, & Martín, 2003). In these species, there may be strong relationships between age and longevity (Reid et al., 2010). The demographic structure of these species contrasts sharply with that of annual species with discrete generations, meaning that evidence of female choice for older males from the former should not be generalized to indicate its likely prevalence in the latter.

Empirical analyses on the effect of male age on female mate choice provide conflicting results. In the laboratory, there are studies showing female preference for older (Avent, Price, & Wedell, 2008; Somashekar & Krishna, 2011), intermediate (Jones, Balmford, & Quinnell, 2000; Jones & Elgar, 2004; Liu, Xu, He, Kuang, & Xue, 2011) and younger males (Prokop, Stuglik, Żabińska, & Radwan, 2007), and even no preference for any particular age of male (Gasparini, Marino, Boschetto, & Pilastro, 2010; Martin, Leugger, Zeltner, & Hosken, 2003). Studies in natural or seminatural populations are scarcer, and report preferences for older males (López et al., 2003; Simmons & Zuk, 1992). Studies on the effect of age on fertilization and embryo or offspring viability are more consistent in their findings. Negative effects of male age on fitness are found both in the laboratory (Jones & Elgar, 2004; Jones et al., 2000; Liu et al., 2011; Preston et al., 2015; Priest, Mackowiak, & Promislow, 2002) and in natural or seminatural environments (Richard, Lecomte, De Fraipont, & Clobert, 2005; Schroeder, Nakagawa, Rees, Mannarelli, & Burke, 2015), and include insect species where the range of possible ages within a cohort is just a few weeks or even days (Jones & Elgar, 2004). A positive effect has been reported by Avent et al. (2008), although they argued that their results were best explained by direct benefits to females due to older males transferring greater quantities of sperm and accessory gland proteins.

Crickets have been proposed as a potential model system for the study of interactions between sexual selection and senescence (Archer & Hunt, 2015). Males sing to attract females and females can discriminate between males based on their calls (Archer & Hunt, 2015; Fitzsimmons & Bertram, 2011; Jacot, Scheuber, & Brinkhof, 2007; Verburgt, Ferreira, & Ferguson, 2011). However, opposing results in relation to female preferences for male age have been found even within the same species: in the cricket *Gryllus bimaculatus*, males' songs declined in energetic quality as they aged and females preferred younger males in a laboratory setting (Verburgt et al., 2011), whereas in a natural population, paired males were older on average than unpaired singing males (Simmons & Zuk, 1992). However, female preferences in relation to male age, and their associated fitness consequences, have never been studied directly in crickets or any other wild insect. We monitored a wild population of the field cricket *Gryllus campestris* in a meadow in northern Spain for 12 annual generations, recording the individual date of adult emergence and death, monitoring behaviours including matings, and building a pedigree across generations. Here we use these data to analyse (1) how reliable male age is as a signal of male longevity, (2) whether female crickets select older or younger males as mates, and (3) whether the sire's age influences female reproductive success.

METHODS

Our WildCrickets project (see WildCrickets.org) has monitored a population of wild *G. campestris* in a meadow in northern Spain for 12 consecutive years, 2006–2017 (Rodríguez-Muñoz, Boonekamp, Fisher, Hopwood, & Tregenza, 2019; Rodríguez-Muñoz, Boonekamp, Liu, Skicko, Fisher, et al., 2019; Rodríguez-Muñoz, Boonekamp, Liu, Skicko, Haugland Pedersen, et al., 2019; Rodríguez-Muñoz, Bretman, Slate, Walling, & Tregenza, 2010). *G. campestris* has a single annual generation; nymphs of both sexes dig burrows in the autumn and overwinter in them, emerging to resume foraging and growth in late spring. The first adults appear in our meadow in late April. A few days after becoming adult, males begin to call to attract females, and both sexes begin moving around the meadow seeking matings. This movement results in frequent switching of burrows, and adults spend 0.45 ± 1.05 days (mean ± SD) between movements. Burrows provide a refuge from predation and bad weather, but are too narrow to allow crickets to mate (Rodríguez-Muñoz, Bretman, & Tregenza, 2011). When two

members of the same sex meet at a burrow, usually one of them immediately leaves, or there is a fight, followed by the loser leaving. When members of the opposite sex meet, fights are very rare; normally either one of them leaves, or the pair temporarily cohabit at the burrow, often mating repeatedly during this period (Rodríguez-Muñoz et al., 2011). After an average ± SD of 0.64 ± 1.44 days, one of the pair moves away from the burrow; we do not know which individual is responsible for initiating these fissions since a male departure might be provoked by some behaviour by females, but pairs that mate more frequently tend to stay together for longer (Rodríguez-Muñoz et al., 2011). Males do not appear to attempt to prevent females from leaving, suggesting that females remaining at a burrow with a male are choosing to do so. Both sexes frequently have multiple mating partners throughout their lives (Rodríguez-Muñoz et al., 2010), and females store sperm (Tyler, Harrison, et al., 2013; Tyler, Rodriguez-Munoz, & Tregenza, 2013).

The WildCrickets meadow is managed in a similar way every year, with the grass being mowed in mid-March and again in July or August. Between August and March, the grass is kept short with additional mowing. Weekly searches for burrows are made from February until the end of the breeding season sometime in July, when the last adult cricket dies. Each burrow is flagged with a unique number that will identify it for the whole breeding season. By mid to late April, usually before the adults start to emerge, we install up to 133 infrared day/night cameras. The cameras use motion-activated digital video recording software (i-Catcher, i-codesystems.co.uk) to continuously record the activity around each burrow entrance onto servers housed in a building adjacent to the meadow.

A few days after each individual emerges as an adult, we trap it in its burrow and glue a PVC tag onto its pronotum. The tag has a unique one- or two-character code allowing us to identify the individual on video. We also collect a drop of haemolymph (sampled by piercing the membrane at the hind leg joint) and a small piece of the tip of one of the hind legs. These samples are later used to provide individual DNA profiles (see below). Marked and sampled crickets are weighed, photographed and released back into the same burrow from which they were collected.

Occupied burrows sometimes outnumber cameras, and adult crickets regularly move around the meadow occupying different burrows. To track crickets at burrows lacking a camera we carry out direct daytime observations every 1–2 days. We record the ID of any adult present or whether a nymph is in residence. This allows us to record accurate adult emergence dates even if burrows are not directly monitored by video at that particular time (nymphs and recently emerged adults rarely move between burrows so the presence of an adult where there was a nymph the day before indicates an emergence). Video watching is still to be concluded for 2 of the 12 years, and pedigree is unavailable for another year, so that the data included in this study are for either 9 or 10 years depending on the specific analysis.

Relationship Between Longevity and Relative Male Age

As discussed above, between-male variation in adult age at any particular point in time arises only as a result of variation in the date of reaching adulthood and is then modulated by differences in adult life span. For convenience we refer to adult age (which we measure from the final moult) and adult life span as age and life span. Variation in emergence date and life span will create a correlation between age and life span within the cohort of individuals alive at any point in time. The strength of this correlation will increase with variance in both life span and emergence date. In our meadow, the adult emergence period across years lasted 21.9 ± 6.5 days (mean ± SD, $N = 10$ years). The interquartile range of emergence date within years was 4.7 ± 1.6 days (mean ± SD, $N = 10$ years). Average male life span including all years was 27.5 ± 18.5 days (mean ± SD, $N = 431$, range 0–76). These distributions show that male age will vary substantially at any given point in the season (Fig. A1).

To determine the extent to which females could use a male's age to predict how long he will live, we tested the relationship between the mean relative male age of each male and his life span. This approach assumes that females choose a mate from those that are available at any given time. To calculate the relative age of a male on any particular day, we subtracted from his age the mean age of the rest of the males also alive on that day. A male's mean relative age across his life span was then the mean of these daily values across his life span. We analysed the relationship between mean relative male age and life span by first running an overall linear mixed model using lme4 (Bates, Mächler, Bolker, & Walker, 2015), and then an independent linear regression test per year. For the mixed model, individual life span was the response variable, mean relative age a fixed effect and year a random effect. We estimated R^2 following the approach of Nakagawa and Schielzeth (2013). For the independent linear regression tests for every year, we again included individual life span as the response variable and mean relative age as the predictor. The strength of this relationship will determine how effective a female strategy of mating with the relatively oldest males is as a method of selecting the longest-lived males.

Female Preference for Males in Relation to Their Age

Females might use three alternative methods to choose mates based on age differences. If females can identify the age of a male in isolation from other males, they could employ a mating rule that makes them more, or less, likely to mate with a male that they encounter according to his absolute age (as envisaged in Kokko & Lindström, 1996). However, in an annual species where the average age of males increases throughout the season, this type of rule would mean that early in the season females would be unwilling to mate with any male, and later on, all males would be extremely attractive. This is clearly not the situation in our crickets where females begin mating as soon as they are sexually mature. A more appropriate mating rule would be that females are more, or less, likely to mate with a male according to his age relative either to her own age or to the age of other males that are also available to the female at that time. The former possibility would require females to know their own age, whereas the latter would only require females to be able to estimate a male's age relative to other males for which information is available at that time. Females will only directly encounter a small proportion of the male population. However, adult males spend a lot of their time producing calling songs audible at distances several multiples of the length of our meadow. The songs of other species of Orthoptera change as males age (Hartley & Stephen, 1989) and female bush crickets have been shown to discriminate between the songs of males that differ in age (Ritchie, Couzin, & Snedden, 1995). This suggests that females may have the opportunity to sample the male population remotely as well as through direct encounters.

We analysed female choice for each period of uninterrupted male occupancy at a burrow (i.e. if the male left the burrow and returned more than 5 min later, the two periods were considered separately). For each of these observation periods, we calculated relative male age as the difference between the age of the target male (averaged over the period of occupancy) and the median age of all adult males alive within that observation period. Only males whose age was known to within 2 days were used in the analysis.

We examined the relationship between relative male age and female mate choice based on two bivariate response variables: Paired and Mated. Paired was scored as 1 when a female was observed sharing a burrow with a male at any time within an observation period and as 0 when the male was on his own over the whole period. Mated is a subset of the pairing variable, representing whether a mating occurred during a period of pairing (scored as 1) or not (scored as 0). In our study population, sexual activity starts a few days after adult emergence. To remove this latency period, our analyses include only crickets older than 5 days (with age measured from the day of adult emergence). Over the entire period of our study, sexually active males and females were monitored in total for about 150 000 h and 178 000 h, respectively. The portion of time that individuals were unpaired was very similar in both sexes: 73% for males and 72% for females. This provides ample latitude for females to express a choice of pairing with available males or remaining unpaired.

We analysed female preferences by running mixed models for Paired and Mated using lme4 (Bates et al., 2015). For the analyses of Paired, whenever more than one female was seen with a male over the same observation period, we included an extra record per female for that observation period. Crickets often visit the same burrows many times; this increases the likelihood that two individuals meet repeatedly at the same burrow just because that burrow is within their shared home range. To avoid the potential for pseudoreplication created by this spatial effect, we only included the first observation period of each pair at any particular burrow in our analysis. For the analysis of Mated, we included all periods when a pair were seen together, regardless of whether it was their first encounter at any given burrow or not. Similar to the Paired analysis, when a male mated with more than one female over the same observation period, we included an extra record per female in the data set for that observation period. We included relative male age and the duration of the observation period as fixed effects, and male identity, burrow identity and year as random effects.

Genetic Analyses and Parentage Assignment

We performed genetic profiling with microsatellite loci to conduct parentage analysis. Previous genotyping in the WildCrickets project used 14 loci, and the details of DNA extraction and genotyping are described elsewhere (Bretman, Rodriguez-Munoz, Walling, Slate, & Tregenza, 2011; Rodríguez-Muñoz et al., 2010). These loci were originally developed in a related species, *G. bimaculatus*, and a number of them had null alleles segregating at high frequency in *G. campestris* (Gbim21) or alleles that were sex linked (Gbim59, Gbim71), making them unsuitable for parentage analysis. Therefore, we retained only 11 of the loci in this study (Gbim4, Gbim15, Gbim26, Gbim29, Gbim33, Gbim49, Gbim52, Gbim53, Gbim57, Gbim66 and Gbim72). Furthermore, for cohorts 2010 onwards loci Gbim26, Gbim29, Gbim33, Gbim53, Gbim66 and Gbim57 were replaced by a set of 10 new loci, identified from RNAseq data generated from *G. campestris* individuals from the WildCrickets meadow. These loci, termed L2590, L5292, L6980, L8550, L9077, L9737, L26448, L26654, L29153 and L30428, are all autosomal and are not segregating for high-frequency (>0.1) null alleles. Details of microsatellite discovery and genotyping are provided in the Appendix. Genotyping was performed on an ABI3730 capillary sequencer, using standard protocols (Ball et al., 2010) and scoring was performed using GeneMapper v3.7 software.

Parentage analysis was performed using genotype data combined with spatial and mating data in a Bayesian framework using the MasterBayes package (Hadfield, Richardson, & Burke, 2006; Koch, Hadfield, Sefc, & Sturmbauer, 2008). This program allows the simultaneous use of both genetic and phenotypic data to infer the pedigree, improving the statistical power of pedigree reconstruction (Hadfield et al., 2006). We used three sources of phenotypic information from our video and direct observations, all relating to the likelihood that a male and a female mated to produce offspring observed in the following year: (1) the Euclidean distances between the average location of a male and a female during a breeding season; (2) whether or not a male and a female were ever observed together ('paired'); and (3) whether or not a male and a female were ever observed to mate.

When using genetic data to infer parentage it is important to consider genotyping error rate (Hadfield et al., 2006; Marshall, Slate, Kruuk, & Pemberton, 2003; Wang, 2004). The majority of pedigree inference programs allow an estimate of error rate to be included in the model. However, MasterBayes allows simultaneous estimation of the genotype of an individual, the pedigree and the relationship between phenotypic data and the pedigree. This allows the genotyping error rate to be estimated rather than assumed and should increase statistical power to reconstruct the pedigree (Hadfield et al., 2006; Koch et al., 2008).

This comes at the cost of a considerable increase in the computational complexity of the models to be estimated (Koch et al., 2008). To accommodate this, we estimated the pedigree on a year by year basis rather than as a single run (single year runs took ca. 30 days running as the only process on a desktop with a 2.0 GHz intel Xeon processor). This is appropriate here as field crickets are annual and thus generations are not overlapping. Finally, the size of the unsampled population can have a considerable influence on the power of pedigree reconstruction and this parameter can also be estimated simultaneously with the pedigree in MasterBayes (Koch et al., 2008). Thus, in assigning parentage we simultaneously considered genetic and phenotypic data and estimated the genotyping error rate and the unsampled population size.

The exact details of the models run varied from year to year (Table A1), but all models were run for long enough and with a large enough interval between samples to minimize autocorrelation in parameter estimates, assessed from plots of model outputs and ensuring autocorrelation between successive samples was low (<0.1). Phenotypic information was considered for all years apart from 2015 as these data have not yet been collated. Genotypes and genotyping error rates were estimated for all models apart from the models estimating parentage for 2013 and 2014. For these years, the models estimating genotypes showed poor mixing (high autocorrelation between successive saved samples of estimates of the phenotypic effects in the model; Table A2). This possibly results from assignment rates in these 2 years being very high; the estimated unsampled population size of male parents was 0.116 (95% confidence interval, CI = 0.0014–1.61) in 2013 and 0.356 (95% CI = 0.0028–2.50) in 2014. As a result, any update to the genotype of an individual during model estimation resulted in a worse fit to the data, constraining the parameter space available for the model to sample. We therefore ran the models for 2013 and 2014 assuming fixed genotypic error rates per locus. A thousand draws of the pedigree were saved for each year and the modal paternity and maternity assignment for each individual were used to assign parentage. Estimates of genotyping error rates for each microsatellite marker are given in Tables A3 and A4.

Effect of Male Age on Offspring Production and Fitness

Female crickets mate and lay eggs throughout their adult lives. They usually mate with more than one male, with separate periods of pairing and mating with a particular partner. To assess the effect of male age on reproductive success, we tested whether a pair's likelihood of producing adult offspring was related to the relative

age of the sire over the period when the pair were observed to mate. This approach of considering each temporary pairing that a male engages in during his life is necessary in order to track the offspring that he may sire in relation to his age. For pairs that mated repeatedly over a period of several days (often including several paired observation periods), we calculated mean relative sire age over the period between the first and the last observed matings. We assessed the possibility that age has a quadratic rather than a linear relationship to adult offspring production by introducing a quadratic term in the analysis. Also, because field cricket females are usually polyandrous, sperm competition can influence fertilization success. Evidence from the closely related *G. bimaculatus* indicates that there is no sperm precedence in relation to mating order in field crickets (Bretman, Newcombe, & Tregenza, 2009; Bretman, Wedell, & Tregenza, 2004). Therefore, we accounted for this effect by including a term that weighted the number of matings of a particular pair relative to the total number of matings observed for the female of that pair. Each pair provided a bivariate variable indicating whether they contributed any offspring to the following generation (1) or not (0). Because females that did not have any offspring are uninformative in relation to the relative success of the males with whom they mate, we only included pairs where the female produced at least one offspring. We ran this analysis by building a full model including the proportion of matings for that pair (PropMatings) and the mean sire age (both as a linear and a quadratic term) as fixed effects, and sire identity and year as random effects. We then compared the full model with three simpler models that excluded, in this order, the quadratic sire age term, the proportion of matings and the year. We based the comparison on the difference in the Akaike information criterion (AIC) values among models, with differences smaller than seven indicating a similar model fit (Burnham, Anderson, & Huyvaert, 2011).

To explore potential long-term effects of male age on offspring fitness, we repeated this analysis but using the production (or not) of grand-offspring as the response variable. We restricted our analysis to only those pairs that produced adult offspring and used the model with the best fit in the previous analysis.

Ethical Note

The crickets used in this study are removed from the meadow for a period of a maximum of a few hours during which time we take a small haemolymph sample and attach a plastic tag by gluing it to the pronotum. Observations of individuals immediately after these procedures indicate that they exhibit normal behaviours within a few minutes of being released, and as far as we know, crickets have never died as a result of any of the procedures used in this study. Our tagged crickets live out their natural lives in the meadow.

RESULTS

Relationship Between Longevity and Relative Male Age

We found a positive relationship between relative male age and longevity ($t = 5.88$, $P < 0.001$, $N = 431$), but the effect was small, with a marginal R^2 of 0.064 (Fig. 1). The independent least squares regressions within years were nonsignificant in most cases, with R^2 values ranging from 0 to 0.262, with a mean of 0.091 (Table 1).

Female Preference for Older Males

Relatively older males attracted more females, but once paired they were less likely to mate. Both relative male age and the duration of the observation period had a positive relationship with the probability of sharing a burrow with a female (Fig. 2a, Table 2). In contrast, although the length of time a pair stayed together was positively associated with their probability of mating, there was a significant negative relationship between relative male age and the probability of mating at least once (Fig. 2b, Table 3). Among the random effects affecting the probability of being paired, burrow identity explained over seven times more variance than male identity, while year had a very small effect (Table 2). However, the effect of burrow identity on the probability of mating once a pair met decreased to about half of the effect of male identity (Table 3).

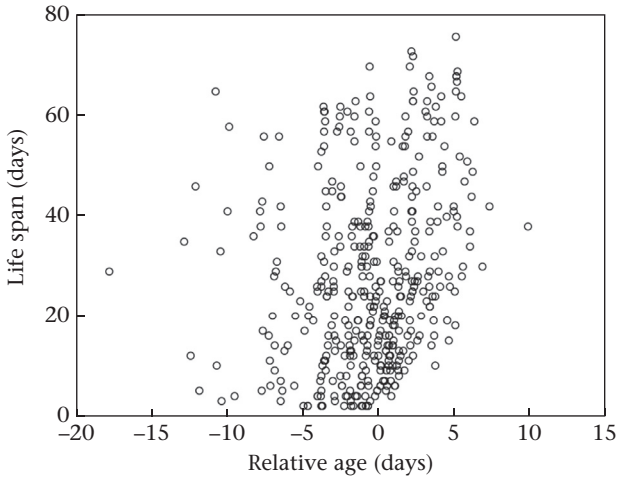

Figure 1. Relationship between relative age over the life of each individual male and his life span in a wild population of *Gryllus campestris*. The bias in favour of males of younger relative ages (more than half the points are relative ages <0) reflects the fact that younger males are more numerous in the population because older males have to be young males earlier in their lives, but young males do not all go on to be old males. The age of a male, relative to the other males alive at any point in time, is only a very weak predictor of his longevity.

Parentage Assignment

The phenotypic data included in the pedigree reconstruction models were generally informative. In the final model, whether a male and female were observed together or not positively influenced their probability of producing offspring together across all years (Table A5). If a male and female were also observed to mate this additionally increased their probability of producing offspring together, although this increase was less than having been seen together in the first place (Table A5). The mean Euclidean distance between a male and a female's burrow also predicted the likelihood of these two individuals producing offspring together, with closer

Table 1
Results of the linear regression analyses between mean relative male age and life span per year in a wild population of *Gryllus campestris*

Year	Coefficient	*P*	R^2	*N*
2006	0.130	**0.007**	0.117	61
2007	0.110	**<0.001**	0.262	47
2008	0.029	0.443	0.055	13
2009	0.050	**0.018**	0.092	61
2010	0.075	0.056	0.171	22
2011	0.070	**0.046**	0.072	56
2012	0.002	0.977	0.000	20
2013	0.026	0.079	0.034	92
2015	0.043	0.124	0.074	33
2016	0.033	0.392	0.031	26

Coefficients with significant *P* values are highlighted in bold.

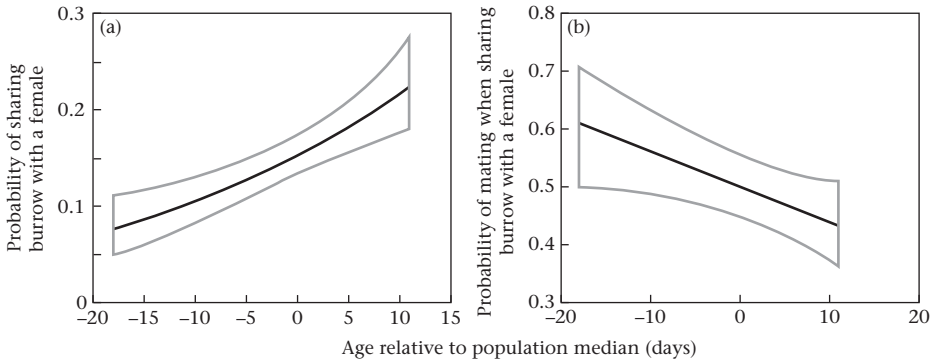

Figure 2. Relationship between relative male age and the probability of the male (a) pairing, that is, sharing his burrow with a female during each period when that male was monitored, and (b) getting at least one mating for every monitored period when he was found sharing a burrow with a female, in a wild population of *Gryllus campestris*. Black lines show the prediction from a linear mixed binomial model, including relative male age and duration of the monitored period as fixed effects, and male identity, burrow and year as random effects. Grey lines show the 95% confidence intervals. (a) $z = 3.868$, $P < 0.001$; (b) $z = -2.276$, $P = 0.023$; see Tables 2 and 3).

neighbours being more likely to have offspring in all years apart from 2007.

Using the modal parentage assignment for each individual, maternity was assigned to a sampled individual in the population for 1267 of 1465 individuals (86% of the population) and paternity to a sampled individual for 1380 individuals (94% of the population). The median confidence of maternity assignments to known individuals was 98.7% (interquartile range, IQR = 30.4; Fig. A2) and in paternity assignments was 98.9% (IQR = 32.1; Fig. A2). A number of individuals were assigned an unsampled parent as the most likely parent (Table A5, Fig. A2), with the size of the unsampled population varying across years, but generally being low.

Effect of Male Age on Offspring Production and Fitness

The best fitting model included the proportion of matings a male got from his partner, but not the quadratic term of relative male age (Table A6). Having a higher proportion of matings with a female increased the probability of producing offspring but being relatively older did not (Table 4). Offspring fitness (the probability that a sire had grand-offspring) was also unrelated to relative male age (Table 5). For offspring fitness, year explained much more variance than male identity because of large differences in population size between years (Table 5).

DISCUSSION

Exactly how females choose among potential mates is a challenging question in its own right. The females in our meadow physically encounter a maximum of a few males a day, but may hear the calls of every singing male in the population. We therefore characterized females as choosing their mates from among all the males alive at any particular time. When we examined the cohorts of males alive each day, we found an overall positive relationship between the longevity of each male and his age relative to the other males in his cohort. However, this relationship was very weak; in 6 of 10 years there was no statistically significant relationship at all (Table 1), and the average proportion of the variance in male longevity predicted by relative age was 0.091. This number represents an absolute upper limit on the power of female choice to select for longevity since it assumes that females can accurately determine male age and can choose the oldest male from all the males alive on any day. This assumption will very rarely be met in any system. In our meadow, females can gather information about males through their calls, but they can only actually mate with

Table 2
Analysis of the relationship between relative male age (as compared to the mean age of the other males in the population) and the probability of cohabiting with a female at any time within each period of continuous observation of a male at a burrow

Fixed effects	Estimate	SD	*P*	Random effects	Variance	SD	*N*
Intercept	−2.701	0.092	**<0.001**	Burrow	1.244	1.115	1 055
Relative age	0.043	0.011	**<0.001**	Individual	0.163	0.403	390
Stay duration	0.628	0.023	**<0.001**	Year	0.012	0.111	10
N	9914						

Results of a mixed model using the lme4 R package with a binomial error distribution. The model includes relative male age and the duration of the monitored period as fixed effects, and year, burrow and male identities as random effects. Coefficients with significant *P* values are highlighted in bold.

Table 3
Analysis of the relationship between relative male age (as compared to the mean age of the other males in the population) and the probability of mating at least once within each period when a male was monitored while sharing a burrow with a female

Fixed effects	Estimate	SD	*P*	Random effects	Variance	SD	*N*
Intercept	−1.518	0.128	**<0.001**	Burrow	0.089	0.299	630
Relative age	−0.025	0.011	**0.023**	Individual	0.182	0.427	313
Stay duration	0.696	0.030	**<0.001**	Year	0.083	0.288	10
N	4528						

Results of a mixed model using the lme4 R package with a binomial error distribution. The model includes relative male age and the duration of the observation period as fixed effects, and year, burrow and male identities as random effects. Coefficients with significant *P* values are highlighted in bold.

Table 4
Analysis of the relationship between relative male age (as compared to the mean age of the other males in the population) and the probability of having at least one offspring

Fixed effects	Estimate	SD	*P*	Random effects	Variance	SD	*N*
Intercept	−0.817	0.297	**0.006**	Individual	0.236	0.486	143
Male age	−0.188	0.118	0.110	Year	0.306	0.553	7
Proportion matings	1.277	0.371	**0.006**				
N	403						

Results of a mixed model using the lme4 R package with a binomial error distribution. The model includes relative male age and the proportion of matings of the target male (from among the total number of matings of his partner) as fixed effects, and year and male identity as random effects. Coefficients with significant *P* values are highlighted in bold.

males that they encounter. Our observations reveal that only a small proportion of individuals ever actually encounter one another (Fisher, Rodríguez-Muñoz, & Tregenza, 2016a; 2016b), placing severe constraints on the capacity of females to select long-lived males based on their relative age.

The extent to which female crickets are able to detect male age is unknown; Verburgt et al. (2011) reviewed studies of the effect of age on calling song in crickets, finding evidence of changes in at least one calling song parameter with age in four of 14 studies. These included Jacot et al.'s (2007) study of *G. campestris*, which identified a difference in carrier frequency of just under 5% between 8-day-old and 30-day-old males, with 19 of 32 males showing a decrease in carrier frequency and eight of 32 males showing an increase. Our population of crickets would appear to be a fairly typical annual insect in relation to the parameters we investigated. Most annual insects have a breeding period lasting for a few weeks or months and females may encounter hundreds of males in their lifetimes, but would very rarely have the opportunity to compare the ages of a large number of males. Given the multiplication of all these factors, it is difficult to avoid the conclusion that female mating preferences are unlikely to exert significant selection in relation to male age in wild insects.

We found that on any given day, older males in the population were more likely to be sharing their burrow with a female than were younger males. Since it appears that male age cannot be used by females to estimate longevity, it seems likely that this effect relates to some other characteristic of males which correlates with their relative age. There is an obvious temptation to speculate that this may be a general reflection of the male's quality. However, such a conclusion is tempered by our finding of a negative relationship between a male's age and the probability that he actually mates with the female with whom the burrow is shared (Fig. 2b, Table 3), which is more consistent with a reproductive advantage for younger males. Our Bayesian parentage assignment approach found that, when burrow sharing is taken into account, adding whether or not a pair actually mated to the hierarchical model only marginally improved the fit. However, this probably reflects the fact that pairs that share a burrow generally mate, and that when the number of burrows was larger than the number of cameras, we could collect data only on whether a pair were together at a burrow and not whether they had mated.

Table 5
Analysis of the relationship between relative male age (as compared to the mean age of the other males in the population) and the probability of having at least one grand-offspring

Fixed effects	Estimate	SD	*P*	Random effects	Variance	SD	*N*
Intercept	−1.176	1.089	0.280	Individual	0.339	0.583	89
Male age	−0.393	0.296	0.184	Year	7.300	2.702	7
N	174						

Results of a mixed model using the lme4 R package with a binomial error distribution. The model includes relative male age as a fixed effect, and year and male identity as random effects.

Burrow identity was a strikingly better predictor of whether a male was paired or not than was his own identity. Burrows were monitored at a similar rate irrespective of their spatial location (and matings are very clearly identifiable) so this finding is not an artefact of differences in our capacity to observe burrows. The large effects of burrow on pairing success suggest that burrow characteristics could be a more important determinant of reproductive success than some aspects of the phenotype including a male's age. This corresponds with Niemelä and Dingemanse's (2017) observation that burrow identity has a large effect on male behaviour. We can only speculate about the burrow features that females might be attracted to, with candidates including spatial location, aspect, physical dimensions and the appearance of the burrow entrance.

We found no relationship between how old a male was when he mated with a particular female and the number of offspring or grand-offspring he contributed to subsequent generations. These analyses were less powerful than others in our study: only 143 males contributed data to the analysis of number of direct offspring and only 89 to the analysis counting grand-offspring. Nevertheless, the fact that there was no hint whatsoever of an effect in either direction suggests that the age of a male when a female mates with him does not have a strong effect on his success in fertilization or the subsequent survival and reproduction of his offspring.

Overall, our observation of relationships between male age and measures of pairing and mating do not provide convincing evidence of female preference for older males in our system. This is presumably why no evidence for increased reproductive success of older males in terms of offspring was apparent. Our study demonstrates that although, in theory, females could gain information about a male's life span from his relative age, in natural insect populations, particularly of annual species, it seems unlikely that a sufficiently reliable signal of longevity is available to females. It is possible that the preferences of females for older males identified in a small proportion of laboratory studies of crickets (Verburgt et al., 2011) reflect the slightly greater correlation between age and longevity expected in multivoltine species. However, this increase (over the very weak correlation we observed) is not expected to be large, suggesting that nonadaptive explanations for female preferences for older males deserve consideration.

Data Availability

All data are available from the authors upon request, including a fasta file of all the contigs used in microsatellite discovery.

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Appendix

Microsatellites were mined from a previously generated 454 transcriptomic sequencing data set of 430 867 reads, using cDNA obtained from two pools of whole males and females. Data were cleaned and assembled using Seqman NGen 2.0.0 (DNASTAR, Madison, WI, U.S.A.) using the Assembly Parameters: Match Size = 31, Match Spacing = 10, Minimum Match Percentage = 90, Match Score = 10, Mismatch Penalty = 20, Gap Penalty = 30 and Max Gap = 15. Microsatellite discovery was only performed on the set of 17 106 assembled contigs, which had a total length of 16 023 190 bp. The contigs were obtained from a total of 262 830 reads.

Microsatellite repeats were detected using a modified version of Sputnik (http://wheat.pw.usda.gov/ITMI/EST-SSR/LaRota). Sputnik parameter settings were − u 2 − v 5 − s 20 − p − L 20, meaning that motifs between 2 and 5 base pairs were reported, provided they were at least 20 bp long and had a score of at least 20. Sequences with a repeat purity of at least 90% were retained. This process yielded a total of 219 unique microsatellite loci (72 dinucleotides, 93 trinucleotides, 35 tetranucleotides and 19 pentanucleotides).

PCR primers were designed for 27 loci, using Primer 3 (Rozen & Skaletsky, 2003). PCR products were tested in a panel of 24 crickets. Loci that failed to produce PCR products, that appeared to be monomorphic in the test panel or were hard to score reliably were excluded from downstream analyses. A set of 10 loci were retained and scored in all cohorts. The microsatellites in the new panel of loci (Table A7) were labelled Lxxxx where xxxx represents the contig that the microsatellite was mined from. A fasta file of all the contigs is available on request.

Each PCR reaction contained 1 μl of air-dried DNA at a concentration of approximately 10 ng/μl, 1 μl of primer mix (fluorescently labelled forward and reverse) at 0.2 μM and 1 μl of QIAGEN multiplex PCR mix (QIAGEN Inc, Manchester, U.K.). The PCR program had an initial denaturation step at 95 °C for 15 min, followed by 45 cycles of 30 s at 94 °C, 90 s at 57 °C and 60 s at 72 °C, followed by a final extension step at 60 °C for 30 min. PCR products were diluted 1:4000 with double-deionized water, before resolving them on an ABI 3730 48-well capillary DNA Analyser (Applied Biosystems, Foster City, CA, U.S.A.). Allele scoring was performed using GeneMapper v3.7 (Applied Biosystems, CA, U.S.A.). The distribution of microsatellite allele lengths was examined using the R package Msatallele (Alberto, 2009), to ensure that binning of estimated fragment lengths (given to two decimal places) to different alleles (scored as integers) was consistent. All individuals that possessed alleles that differed by 1 bp were genotyped at least twice, along with additional control individuals of known genotype, to check for allele sizing errors. Observed and expected heterozygosities were

Table A1
Details of the models run for each year of pedigree reconstruction

Total number of iterations	Burn-in	Thinning interval	Estimate genotyping error rate	Estimate phenotypic data	Year
65 000 000	15 000 000	50 000	Yes	Yes	2007, 2009, 2011, 2012
65 000 000	15 000 000	50 000	Yes	No	2015
13 000 000	3 000 000	10 000	Yes	Yes	2008
130 000	30 000	1000	No	Yes	2013
650 000	150 000	5000	No	Yes	2014

Table A2
Autocorrelation between successive samples of the estimate of the effect of phenotypic data on the probability of a male and female mating.

Year	Not estimating genotyping error rate				Estimating genotyping error rate			
	Sample	Paired	Mated	Distance	Sample	Paired	Mated	Distance
2013	0	1	1	1	0	1	1	1
	1	0.0328	0.0380	0.0254	1	0.1599	0.0740	0.1469
	5	−0.0247	−0.0143	0.0316	5	0.1042	0.1100	0.1015
	10	0.0521	−0.0007	0.0116	10	0.1029	0.0515	0.0570
	50	0.0662	0.0163	−0.0255	50	0.0198	−0.0107	0.0362
2014	0	1	1	1	0	1	1	1
	1	−0.0468	−0.0206	0.0301	1	0.3597	0.1756	0.1934
	5	−0.0224	−0.0084	0.0028	5	0.2753	0.0589	0.1174
	10	−0.0059	−0.0055	0.0082	10	0.3078	0.1182	0.1279
	50	0.0522	0.0243	0.0187	50	0.1978	0.0486	0.0813

Data are presented from runs assigning parentage to individuals from 2013 and 2014 either without (left) or with (right) genotyping error rates being estimated. The autocorrelation between successive samples when estimating genotyping error rate is considerably higher than when not estimating error rate, suggesting models estimating error rate are not mixing well. All analyses in the paper are based on models that do not estimate genotyping error rates for these two 2 years only.

Table A5
Parameter estimates for the pedigree reconstruction models

Parameter	Year	Estimate	95% CI
Unsampled female population size	2007	12	6.27–18.7
	2008	0.554	0.002–17.5
	2009	1.250	0.36–3.07
	2011	25.9	16.8–40.9
	2012	17.5	4.6–39.6
	2013	0.163	0.001–2.660
	2014	12.3	6.4–16.8
	2015	63.9	27.3–121.9
Unsampled male population size	2007	1.87	0.13–6.45
	2008	0.125	0.001–9.141
	2009	0.790	0.181–1.636
	2011	23.6	14.8–34.1
	2012	0.788	0.010–11.199
	2013	0.116	0.001–1.61
	2014	0.356	0.003–2.50
	2015	18.9	1.6–62.6
Paired	2007	3.51	3.24–4.07
	2008	2.96	2.14–3.99
	2009	3.37	2.76–4.10
	2011	2.10	1.46–2.54
	2012	2.49	1.74–3.39
	2013	2.49	1.87–3.09
	2014	2.51	2.29–2.80
	2015	NA	NA
Mated	2007	0.0470	8.38×10^{-5}–0.0885
	2008	0.0572	−0.0683 to 0.272
	2009	−0.0441	−0.127 to 0.0952
	2011	0.400	0.149–0.554
	2012	0.157	−0.062 to 0.376
	2013	0.0445	−0.0167 to 0.108
	2014	−0.0023	−0.0627 to 0.0407
	2015	NA	NA
Distance	2007	0.00697	−0.0241 to 0.0304
	2008	−0.0807	−0.1384 to −0.0081
	2009	−0.0364	−0.0077 to 0.0038
	2011	−0.0996	−0.130 to −0.0193
	2012	−0.0797	−0.131 to −0.0193
	2013	−0.203	−0.249 to −0.126
	2014	−0.135	−0.159 to −0.112
	2015	NA	NA

CI: confidence interval. The parameters Paired and Mated are estimated as the log odds ratio, $\log(\theta/1-\theta)$, where θ is the probability of a male and a female producing offspring together if they expressed the parameter of interest versus if they did not. The parameter Mated is conditional on the pair having been seen together.

calculated and null allele frequencies estimated using CERVUS v3.0.3 (Kalinowski, Taper, & Marshall, 2007), and deviations from Hardy–Weinberg Equilibrium (HWE) and linkage equilibrium were assessed with GENEPOP v4.0.10 (Rousset, 2008; Raymond & Rousset, 1995; Table A8).

Table A3
Estimates of genotyping error rates for each microsatellite marker from models estimating this parameter

Microsatellite	Year	Error	Posterior mode	Lower 95% CI	Upper 95% CI	Error	Posterior mode	Lower 95% CI	Upper 95% CI
Gbim26	2007	E1	2.06E-01	1.48E-01	2.50E-01	E2	8.31E-04	5.55E-06	1.42E-02
Gbim29	2007	E1	2.46E-02	9.61E-03	5.30E-02	E2	6.29E-03	8.72E-05	2.15E-02
Gbim33	2007	E1	1.76E-03	7.88E-06	1.66E-02	E2	8.85E-03	8.86E-05	2.71E-02
Gbim53	2007	E1	4.85E-02	3.41E-02	7.65E-02	E2	4.58E-02	2.41E-02	6.88E-02
Gbim57	2007	E1	1.38E-01	9.11E-02	1.74E-01	E2	3.76E-03	3.69E-04	1.76E-02
Gbim66	2007	E1	1.43E-01	1.06E-01	1.87E-01	E2	1.28E-02	1.31E-04	2.47E-02
Gbim49	2007	E1	1.81E-02	4.81E-04	5.16E-02	E2	4.40E-03	3.13E-05	1.40E-02
Gbim52	2007	E1	4.51E-02	2.88E-02	9.47E-02	E2	1.83E-03	3.09E-05	1.43E-02
Gbim72	2007	E1	1.02E-02	4.52E-05	4.75E-02	E2	5.26E-04	4.11E-06	8.78E-03
Gbim04	2007	E1	2.26E-02	2.59E-03	3.88E-02	E2	2.41E-03	8.12E-05	1.43E-02
Gbim15	2007	E1	1.93E-02	1.88E-03	3.48E-02	E2	1.19E-02	2.42E-03	2.61E-02
Gbim26	2008	E1	1.85E-01	1.31E-01	2.38E-01	E2	2.02E-04	1.16E-06	1.30E-02
Gbim29	2008	E1	4.45E-04	3.65E-06	1.73E-02	E2	8.63E-03	7.24E-06	2.72E-02
Gbim33	2008	E1	1.94E-02	2.38E-03	4.40E-02	E2	1.10E-03	3.50E-05	3.06E-02
Gbim53	2008	E1	2.02E-02	1.81E-03	4.26E-02	E2	9.01E-02	4.75E-02	1.22E-01
Gbim57	2008	E1	1.28E-01	8.71E-02	1.81E-01	E2	9.53E-04	3.84E-07	2.56E-02
Gbim66	2008	E1	1.04E-01	5.59E-02	1.37E-01	E2	1.09E-02	7.54E-04	4.01E-02
Gbim49	2008	E1	2.79E-02	9.98E-05	8.07E-02	E2	4.86E-03	1.72E-06	1.93E-02
Gbim52	2008	E1	2.88E-02	1.40E-03	6.70E-02	E2	5.26E-04	7.27E-06	1.57E-02
Gbim72	2008	E1	3.41E-03	6.40E-05	5.86E-02	E2	1.12E-04	2.09E-06	1.06E-02
Gbim04	2008	E1	4.27E-04	4.74E-06	2.29E-02	E2	5.43E-04	1.44E-06	1.34E-02
Gbim15	2008	E1	2.96E-02	1.25E-02	7.40E-02	E2	1.01E-03	7.12E-06	1.69E-02
Gbim26	2009	E1	1.73E-01	1.31E-01	2.42E-01	E2	1.48E-02	2.80E-03	3.25E-02
Gbim29	2009	E1	1.11E-02	4.71E-05	3.21E-02	E2	1.44E-03	1.15E-06	1.26E-02
Gbim33	2009	E1	7.22E-04	5.52E-07	1.09E-02	E2	4.37E-04	1.31E-06	1.20E-02
Gbim53	2009	E1	8.15E-03	8.89E-06	3.85E-02	E2	1.71E-01	1.30E-01	2.14E-01
Gbim57	2009	E1	9.14E-02	5.72E-02	1.41E-01	E2	1.82E-02	6.77E-03	3.88E-02
Gbim66	2009	E1	7.72E-02	4.89E-02	1.26E-01	E2	3.53E-03	4.61E-04	2.03E-02
Gbim49	2009	E1	2.20E-02	2.28E-03	1.05E-01	E2	2.51E-03	2.67E-06	1.46E-02
Gbim52	2009	E1	6.29E-03	3.24E-05	4.28E-02	E2	2.59E-02	1.04E-02	4.55E-02
Gbim72	2009	E1	1.82E-03	5.55E-06	3.61E-02	E2	3.15E-04	5.54E-07	8.27E-03
Gbim04	2009	E1	9.08E-04	6.56E-06	1.45E-02	E2	6.20E-04	2.69E-06	9.05E-03
Gbim15	2009	E1	1.62E-04	3.95E-06	1.34E-02	E2	1.25E-04	1.38E-06	1.04E-02
Gbim49	2011	E1	2.13E-02	2.53E-05	7.23E-02	E2	1.11E-04	2.13E-06	5.67E-03
Gbim52	2011	E1	4.08E-02	1.75E-02	8.15E-02	E2	6.73E-04	5.27E-06	1.00E-02
Gbim72	2011	E1	2.24E-02	7.56E-03	5.11E-02	E2	3.06E-04	6.26E-07	5.67E-03
Gbim04	2011	E1	9.44E-03	9.49E-04	2.39E-02	E2	1.71E-03	1.29E-06	1.07E-02
Gbim15	2011	E1	5.70E-03	2.76E-04	2.61E-02	E2	7.02E-04	6.36E-06	1.31E-02
L8550	2011	E1	1.61E-02	2.59E-03	4.41E-02	E2	2.25E-04	1.21E-06	1.23E-02
L9077	2011	E1	2.25E-02	5.31E-03	4.54E-02	E2	1.12E-04	1.77E-07	7.49E-03
L26448	2011	E1	2.13E-02	9.08E-03	3.84E-02	E2	5.79E-03	3.35E-06	1.96E-02
L30428	2011	E1	1.59E-02	8.22E-04	3.13E-02	E2	3.15E-03	9.85E-06	1.61E-02
L9737	2011	E1	7.40E-02	4.62E-02	1.05E-01	E2	8.45E-03	3.65E-04	2.20E-02
L2590	2011	E1	1.72E-02	2.94E-03	3.72E-02	E2	6.89E-03	1.48E-03	2.57E-02
L5292	2011	E1	3.09E-03	7.71E-06	1.52E-02	E2	1.85E-04	2.05E-06	8.92E-03
L6980	2011	E1	8.75E-03	7.69E-05	2.34E-02	E2	4.98E-04	8.92E-07	1.31E-02
L26654	2011	E1	9.29E-03	1.34E-03	3.54E-02	E2	8.23E-04	2.44E-06	1.02E-02
L29153	2011	E1	1.17E-02	1.17E-04	2.28E-02	E2	9.26E-04	1.43E-05	1.23E-02
Gbim49	2012	E1	8.09E-02	3.67E-02	1.57E-01	E2	2.80E-04	7.83E-07	7.55E-03
Gbim52	2012	E1	4.20E-02	4.61E-03	7.55E-02	E2	4.16E-03	3.16E-04	1.57E-02
Gbim72	2012	E1	1.95E-03	3.24E-05	4.59E-02	E2	1.43E-03	7.73E-05	1.01E-02
Gbim04	2012	E1	6.94E-04	2.38E-05	3.26E-02	E2	3.17E-04	7.73E-06	9.27E-03
Gbim15	2012	E1	5.70E-04	1.58E-05	2.53E-02	E2	3.52E-03	2.18E-05	1.78E-02
L8550	2012	E1	5.20E-04	1.26E-05	2.50E-02	E2	5.01E-03	1.78E-04	2.11E-02
L9077	2012	E1	3.11E-02	4.54E-03	5.52E-02	E2	6.01E-03	8.33E-04	1.81E-02
L26448	2012	E1	3.10E-02	1.60E-02	6.25E-02	E2	1.85E-02	1.14E-03	4.40E-02
L30428	2012	E1	4.09E-02	1.51E-02	8.09E-02	E2	1.37E-03	1.15E-05	1.51E-02
L9737	2012	E1	1.26E-01	8.71E-02	1.81E-01	E2	7.46E-02	4.48E-02	1.13E-01
L2590	2012	E1	2.80E-02	7.70E-03	6.59E-02	E2	1.91E-02	6.59E-03	5.16E-02
L5292	2012	E1	6.82E-03	2.00E-04	2.87E-02	E2	3.54E-04	5.69E-06	1.23E-02
L6980	2012	E1	3.78E-03	1.09E-05	2.45E-02	E2	5.62E-03	1.93E-05	2.70E-02
L26654	2012	E1	7.10E-04	5.27E-06	2.40E-02	E2	7.02E-04	7.63E-06	1.50E-02
L29153	2012	E1	8.22E-03	5.91E-06	2.64E-02	E2	2.73E-04	5.66E-06	1.92E-02
Gbim49	2015	E1	1.29E-01	7.92E-02	1.78E-01	E2	5.21E-05	1.43E-06	3.99E-03
Gbim52	2015	E1	5.71E-02	3.18E-02	8.66E-02	E2	8.46E-05	1.22E-06	4.08E-03
Gbim72	2015	E1	2.56E-02	5.55E-05	4.98E-02	E2	3.05E-04	5.65E-07	4.48E-03
Gbim04	2015	E1	5.23E-04	7.29E-06	2.21E-02	E2	4.11E-03	4.38E-04	1.05E-02
Gbim15	2015	E1	7.44E-03	8.65E-04	2.73E-02	E2	2.89E-04	2.73E-07	4.89E-03
L8550	2015	E1	1.49E-02	2.76E-03	3.20E-02	E2	6.16E-04	3.22E-06	9.94E-03
L9077	2015	E1	7.63E-02	4.86E-02	9.76E-02	E2	1.41E-04	8.94E-07	6.60E-03
L26448	2015	E1	9.41E-02	6.64E-02	1.15E-01	E2	3.88E-04	7.78E-06	1.23E-02
L30428	2015	E1	1.17E-04	2.60E-07	8.80E-03	E2	1.69E-03	3.09E-06	1.02E-02

(*continued on next page*)

Table A3 (*continued*)

Microsatellite	Year	Error	Posterior mode	Lower 95% CI	Upper 95% CI	Error	Posterior mode	Lower 95% CI	Upper 95% CI
L9737	2015	E1	1.07E-01	8.24E-02	1.35E-01	E2	2.80E-03	1.77E-04	1.12E-02
L2590	2015	E1	4.23E-02	2.36E-02	6.00E-02	E2	1.56E-02	5.06E-03	2.79E-02
L5292	2015	E1	3.17E-02	1.43E-02	4.90E-02	E2	2.61E-03	4.07E-05	1.16E-02
L6980	2015	E1	1.13E-02	1.11E-03	2.58E-02	E2	9.06E-04	3.67E-05	1.69E-02
L26654	2015	E1	5.16E-03	7.45E-05	1.91E-02	E2	2.49E-03	1.64E-05	1.84E-02
L29153	2015	E1	1.70E-02	6.34E-03	3.64E-02	E2	1.41E-03	3.65E-05	1.25E-02

As described in the Methods, this was done for years 2007–2009, 2011–2012 and 2015. The markers used in 2007–2009 differ from those used in the other years. Estimates are posterior modes and their 95% confidence intervals (95% CI). Genotyping error rates are estimated as two parameters: E1, the probability of heterozygote dropout, and E2, the probability of alleles being mis-scored.

Table A4
Assumed genotyping error rates for years 2013 and 2014 when models estimating error rate failed to mix

Microsatellite	E1	E2
Gbim49	0.08	0.005
Gbim52	0.03	0.005
Gbim72	0.01	0.005
Gbim04	0.01	0.005
Gbim15	0.01	0.005
L8550	0.01	0.005
L9077	0.03	0.005
L26448	0.06	0.005
L30428	0.01	0.005
L9737	0.1	0.005
L2590	0.03	0.005
L5292	0.01	0.005
L6980	0.01	0.005
L26654	0.01	0.005
L29153	0.01	0.005

E1 is an estimate of the probability of heterozygote dropout, and E2 is an estimate of the probability of alleles being mis-scored.

Table A6
Model comparison for the analysis of the relationship between relative male age (as compared with the mean male age in the population) and offspring production

Model	*df*	ΔAIC
Off ~ PropMating + SireAge + SireAge^2 + (1 I ID) + (1 I Year)	6	2
Off ~ PropMating + SireAge + (1 I ID) + (1 I Year)	5	0
Off ~ SireAge + (1 I ID) + (1 I Year)	4	10
Off ~ SireAge + (1 I ID)	3	18

The full model includes the proportion of matings by the male from among those recorded for the female whose offspring production is considered (PropMating), the mean relative male age over the period when he was seen mating with that female both as a linear (SireAge) and a quadratic (SireAge^2) term as fixed effects, and male identity (ID) and year (Year) as random effects. The table shows the difference in the Akaike information criterion, AIC (ΔAIC) for each model as compared to the simplest model with the smallest AIC (fits for models with a ΔAIC <7 are considered equivalent; Burnham et al., 2011). All models were analysed using the lme4 R package (Bates et al., 2015) with a binomial family distribution, the response variable (Off) being whether the pair had adult offspring (1) or not (0) in the following generation.

Table A7
Summary of new (prefix L) and original (prefix Gbim) microsatellite loci

Locus	Repeat motif	Primer sequence	Tm	Expected allele size	Allele size range (bp)	Dye	Multiplex
L2590	AC	F:GCAACATAAATAAAGGTTGCTTCC	60.3	218	204–226	NED	2
		R:TGACGTTGTAGTTGGCGAAG	59.9				
L5292	AC	F:TCAAATTGTTGTGACCCAGTG	59.5	261	252–296	FAM	2
		R:AAACTAGGAATGGCAAACTTCATC	59.9				
L6980	AC	F:CTATATGACCATTTGTTCAGTCTGTTC	59.4	237	220–237	VIC	2
		R:TCCCTACATGCGGTACACTG	59.6				
L8550	ACGG	F:TTGCAGAATTCCCTTGTGG	59.6	236	230–256	FAM	1
		R:AACCAATCCGAGGTGGTAATC	60.1				
L9077	AC	F:TGAGGAAATTGTTGAGTTGGAG	59.2	296	290–331	PET	1
		R:GCCACTCTTCATTAACTTCCTATAAAC	59.2				
L9737	AC	F:ACAGGGAAATATTCTAGGCTTCC	59.1	193	163–216	NED	1
		R:AGTGTAAACAGCATTCATTCAACTAAG	59.3				
L26448	AC	F:GCATGCAAGAAGAGCATGAG	59.7	172	168–235	PET	1
		R:TCATCTGCACAAGCACACAC	59.4				
L26654	AC	F:AGCAAGTTTAAGACAGTCCTGATG	59.0	200	189–208	FAM	2
		R:GGAACTTGGAGCAGAACATAAAG	59.3				
L29153	AC	F:AGCTGGTACTACAACTGCCATTC	59.7	217	213–249	PET	2
		R:TTGTCCAAGATAAGTGGAAACTTG	59.6				
L30428	AC	F:CTCTCAGCACACAGACAATGC	59.6	212	206–223	VIC	1
		R:TGTGGAACTATTCCTTTATCTCACC	59.8				
Gbim04	GT	F:CGACGTATGTAGGCCTGCGG	65.0		205–239	FAM	3
		R:ATCCTACCAACACGGCACGG	65.0				
Gbim15	CA	F:GACTGCGGGTACCCTTGTCG	65.0		167–197	VIC	3
		R:ATCCGGAGCTTCAGCAAGGC	65.0				
Gbim29	$(CA)_3A(CA)_{16}$	F:GATCCATTTCCGCCACTTCG	65.0		270–299	NED	3
		R:ACCATCCGTTCGCTTTCTCG	65.0				
Gbim33	$(GATA)_{14}(GATT)_3$	F:GCTTCAGAAGGCGAAGACACG	65.0		265–347	PET	3
		R:TTGGTGGATTGTGACGATTATTGC	65.0				
Gbim49	GT	F:TTGCCACATCTCCCGAGAAAG	65.0		206–240	FAM	4
		R:TTGGTCCGTGCGTGGTAATTC	65.0				
Gbim52	CA	F:ACACCAGGCGAATGTCGAAAC	65.0		163–178	VIC	4
		R:CCAGACGGGACTTGCTCAAAG	65.0				
Gbim53	$(CT)_4TT(CT)_2TT(CA)_{12}$	F:TCTTTCTTTCTTCACTCTTGACCACTCC	65.0		120–186	NED	4
		R:CGCCATGTGGGATGCTGTAG	65.0				
Gbim72	CA	F:ACCAGGTGAATGTCGGAGCAG	65.0		180–241	PET	4
		R:CAGTGTGGCACCACAGCAATC	65.0				

Motifs, primer sequences, size ranges and PCR conditions are given.

Table A8
Genotyping summary statistics of typed loci (2010 cohorts onwards)

Locus	k	n	H_O	H_E	HWE	$F_{(null)}$
L2590	12	366	0.604	0.602	*	0.009
L5292	8	360	0.747	0.548	***	−0.163
L6980	10	376	0.721	0.729	*	0.004
L8550	9	385	0.579	0.591	NS	−0.001
L9077	8	376	0.668	0.722	NS	0.040
L9737	19	369	0.702	0.865	***	0.104
L26448	19	379	0.821	0.864	NS	0.024
L26654	10	381	0.572	0.577	NS	0.005
L29153	13	376	0.878	0.859	NS	−0.013
L30428	10	385	0.610	0.615	NS	−0.002
Gbim04	11	385	0.735	0.717	NS	−0.014
Gbim15	10	385	0.751	0.738	NS	−0.012
Gbim49	4	391	0.330	0.395	**	0.093
Gbim52	7	391	0.509	0.536	NS	0.026
Gbim72	3	392	0.579	0.499	*	−0.075

k = number of alleles; n = number of genotyped individuals; H_E = expected heterozygosity; H_O = observed heterozygosity; HWE = significance of test for departure from Hardy–Weinberg equilibrium; $F_{(null)}$ is the estimate of null allele frequency, based on an excess of observed homozygotes.
*$P < 0.05$; **$P < 0.01$; **$P < 0.001$.

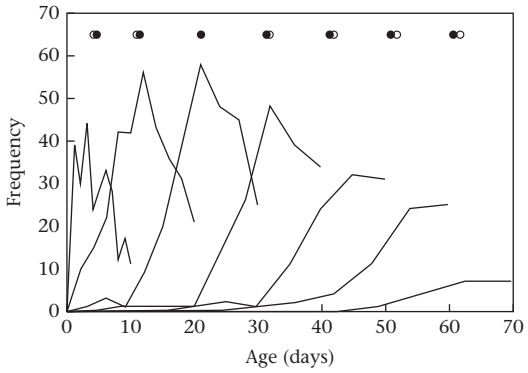

Fig. A1. Age distribution of male crickets over the breeding season at 10-day intervals. Data were pooled over 10 consecutive seasons. Black and white circles show the mean and median age for each interval, respectively.

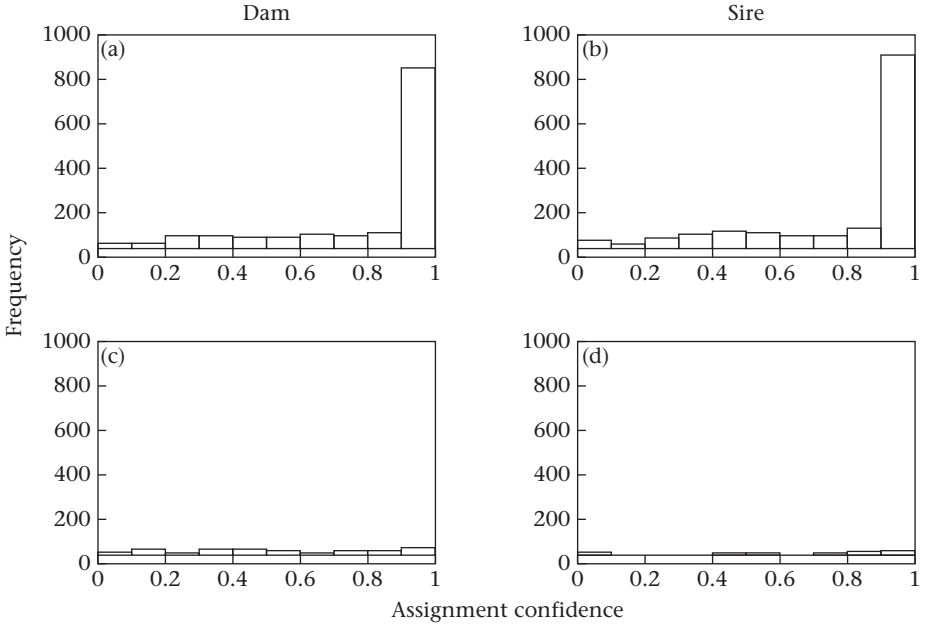

Fig. A2. Distribution of confidence in the assignments of (a, c) mothers (dams) and (b, d) fathers (sires). (a, b) Known dams and sires are assignments made to sampled crickets in the population. (c, d) Unknown dams and sires are assignments where the most likely parent is an individual that was not sampled in the population.